ANTI-INFLAMMATORY AND EPIDERMAL BARRIER PROTECTING ACTIVITIES OF VIRGIN COCONUT OIL

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DEDICATION

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ABSTRACT

Coconut oil (CO) has been used for centuries as skin moisturizers and disinfectants in Southeast Asia. However, the underlying procedure on the skin protecting activities is still elusive. Thus in the present study, the potential anti-inflammatory and skin barrier protecting activities of CO were investigated using *in vitro* and *in vivo* models. The virgin coconut oil (VCO) was extracted using the integrated wet process and its physical, chemical and antioxidant properties were analysed. Furthermore, a comparative study between VCO, refined CO, and main fatty acid derivatives from CO such as myristic and palmitic acid on anti-inflammatory and scratch-wound healing, was tested using *in vitro* assays. In addition, a double-blinded study on the skin barrier recovery activities using a non-invasive tape stripping method was performed on 10 healthy female subjects. The subjects were topically treated with VCO or palm oil (PO). The results revealed that VCO holds the highest amount of lauric acid (50.23 ± 1.22 %) and possesses excellent antioxidant properties (IC_{50}, 2.842 ± 1.14 mg/L) compared to other samples. VCO exhibited a high percentage of cell viability and tolerance to keratinocytes and fibroblasts at concentrations up to 1.0 mg/mL. Treatment with VCO significantly inhibited the reactive oxygen species, tumour necrosis factor-α, and interleukin-6 production. Furthermore, in the scratch-wound healing study, VCO has significantly (p <0.05) enhanced proliferation and migration of fibroblast cells as compared to the untreated control and other fatty acid derivatives. VCO also exhibited the highest percentage of scratch-wound closure (11.65 ± 8.21 %) compared to other samples. VCO also shows a reduction in hyaluronidase enzymes (31.52 ± 4.60 %) that plays a critical role in wound pathogenesis. In the epidermal damage study by tape stripping method, topical application of VCO indicated a significant reduction of transepidermal water loss compared to PO and untreated control (p <0.05). Improvement in skin hydration was also observed in VCO (30 %) and PO (29 %) treated areas. Interestingly, a statistically significant difference was discovered in ceramides and free fatty acids contents on samples treated with VCO and PO as compared to the untreated samples. The ceramide/cholesterol ratio in VCO treated sample was found to be marginally higher compared to PO and untreated samples. From these findings, VCO was found to significantly improve the skin barrier properties through reduction of inflammation, acceleration of wound closure, and balancing of the stratum corneum lipid composition, compared to PO. Taken together, the results of this study demonstrated that VCO might offer great potential as a topical therapeutic agent, as well as in epidermal barrier repair and protection.

ABSTRAK

Minyak kelapa (CO) telah digunakan selama berabad-abad sebagai pelembap kulit dan disinfektan di Asia Tenggara. Walau bagaimanapun, prosedur yang mendasari aktiviti perlindungan kulit masih sukar difahami. Oleh itu, dalam kajian ini, potensi aktiviti anti-radang dan pertahanan kulit oleh CO diselidiki menggunakan model *in vitro* dan *in vivo*. Minyak kelapa dara (VCO) telah diekstrak melalui proses basah bersepadu dan sifat fizikal, kimia dan antioksida VCO telah dianalisa. Selanjutnya, kajian perbandingan antara VCO, CO bertapis dan pecahan asid lemak CO utamanya seperti asid miristik dan palmitik terhadap anti-radang dan penyembuhan luka telah dilakukan secara *in vitro*. Di samping itu, kajian *double-blinded* terhadap aktiviti pemulihan pertahanan kulit menggunakan kaedah pelucutan pita yang tidak invasif dilakukan dengan 10 subjek wanita yang sihat. Subjek dirawat secara topikal dengan menggunakan VCO atau minyak kelapa sawit (PO). Hasil kajian menunjukkan VCO mempunyai jumlah asid laurik tertinggi (50.23 ± 1.22 %) dan mempunyai sifat antioksida yang sangat bagus (IC_{50}, 2.842 ± 1.14 mg/L) berbanding sampel yang lain. VCO menunjukkan peratus *cell viability* dan toleransi yang tinggi terhadap sel keratinosit dan fibroblas pada kepekatan sehingga 1.0 mg/mL. Rawatan dengan VCO dapat menghalang pengeluaran *reactive oxygen species, tumour necrosis factor-α*, dan *interleukin-6*. VCO telah menunjukkan peningkatan yang ketara (p <0.05) di dalam percambahan dan penghijrahan sel-sel fibroblas berbanding sampel yang tidak dirawat dan pecahan asid lemak yang lain di dalam ujian penyembuhan luka. VCO juga menunjukkan peratusan penutupan calar tertinggi (11.65 ± 8.21 %) berbanding sampel yang lain. VCO menunjukkan pengurangan aktiviti enzim hyaluronidase (31.52 ± 4.60 %) yang memainkan peranan penting dalam patogenesis luka. Dalam kajian kerosakan epidermis melalui kaedah pelucutan pita, penggunaan VCO secara topikal menunjukkan pengurangan kehilangan air *transepidermal* yang ketara berbanding dengan PO dan sampel kawalan yang tidak dirawat (p <0.05). Peningkatan dalam penghidratan kulit juga diperhatikan di kawasan yang dirawat VCO (30 %) dan PO (29 %). Menariknya, perbezaan yang ketara secara statistik ditemui dalam kandungan *ceramides* dan asid lemak bebas pada sampel dirawat dengan VCO dan PO, berbanding dengan sampel yang tidak dirawat. Nisbah *ceramide*/kolesterol dalam sampel yang dirawat dengan VCO didapati lebih tinggi berbanding dengan sampel PO dan sampel yang tidak dirawat. Melalui penemuan ini, VCO didapati meningkatkan sifat pertahanan kulit dengan ketara melalui pengurangan radang, mempercepat penutupan luka, dan mengimbangi komposisi lemak *stratum corneum* berbanding dengan PO. Secara keseluruhan, hasil kajian ini menunjukkan bahawa VCO mungkin berpotensi besar sebagai agen terapeutik topikal, begitu juga di dalam pembaikan dan perlindungan epidermis.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

AA	-	Arachidonic acid
AD	-	Atopic Dermatitis
ALA	-	α-linolenic acid
ANOVA	-	Analysis of variance
AP	-	Alkaline phosphatase
AOCS	-	American Oil Chemist Society
AOAC	-	Association of Analytical Communities
APCC	-	Asian Pacific Coconut Community
AsA	-	Ascorbate
ATP	-	Adenosine triphosphate
AU	-	Arbitrary unit
AV	-	Acid value
BCl3	-	Boron trichloride
BHT	-	Butylated hydroxytoluene
CA	-	Caproic acid
CAT	-	Catalase
CCK-8	-	Cell Counting Kit-8
CE	-	Cornified envelope
CER	-	Ceramide
CER [ADS]	-	Ceramide ω-hydroxy fatty acids & dihydrosphingosines
CER [EOS]	-	ω-hydroxy-ceramide
CERNH	-	Ceramide nonhydroxy fatty acids & 6-hydroxysphingosines
CER [NP]	-	Ceramide nonhydroxy fatty acids & phytosphingosines
CERAP	-	Ceramide α-hydroxy fatty acids & phytosphingosines
CerS3	-	Esterified v-hydroxy ceramides
CHOL	-	Cholesterol
CL	-	Caprylic acid
CM1	-	Complete media 1

CM2	-	Complete media 2
CoA	-	Covalent adjustment
CO_2	-	Carbon dioxide
COX	-	Cyclooxygenase
COX-2	-	Cyclooxygenase-2
CP	-	Capric acid
CPD	-	Cyclobutane pyrimidine dimers
CPE	-	Chemical permeation enhancers
CRP	-	C-reactive protein
CSO_4	-	Cholesterol sulphate
$CuSO_4$	-	Copper sulphate
CTL	-	Cytotoxic T lymphocytes
DAG	-	Diacylglycerol
DCF	-	2',7'-dichlorofluorescein
DCF-DA	-	2',7'-dichlorofluorescein-diacetate
DDAB	-	Didecyldimethylammonium bromide
DEX	-	Dexamethasone
DGAT	-	Diglyceride acyltransferase
DHA	-	Docosahexaenoic acid
DNA	-	Deoxyribonucleic acid
DMSO	-	Dimethyl sulfoxide
DMEM	-	Dulbecco’s modified essential medium
DPA	-	Docosapentaenoic acid
DPPH	-	2,2-diphenyl-1-picrylhydrazyl
DRS	-	Diffuse reflectance spectroscopy
DTAB	-	Didecyltrimethylammonium bromide
ECACC	-	European Collection of Authenticated Cell Culture
ECM	-	Extracellular matrix
EFA	-	Essential fatty acids
EIA	-	Enzyme immunoassay
ELISA	-	Enzyme Linked Immunosorbent Assay
ELOVL1	-	Elongase-1

EMW	-	Electromagnetic wave
EPA	-	Eicosapentaenoic acid
ESI	-	Electrospray ionisation
FA	-	Fatty acid
FFA	-	Free fatty acid
FAME	-	Fatty acid methyl esters
FBS	-	Fetal bovine serum
FDA	-	Food and Drug Administration
FLG	-	Filaggrin
FTM	-	Full thickness model
GAPDH	-	Glyceraldehyde 3-phosphate dehydrogenase
GLC	-	Gas/liquid chromatography
GSH	-	Glutathione
GPX	-	Glutathione peroxidase
GR	-	Glutathione reductase
GT	-	Glyceryl trioleate
HA	-	Hyaluronic acid
HAase	-	Hyaluronidase
HABSI	-	Hospital-acquired blood stream infections
HaCaT	-	Immortalised human keratinocytes
HASs	-	Hyaluronic acid synthases
HCl	-	Hydrochloric acid
HIV	-	Human immunodeficiency virus
HDL	-	High-density lipoprotein
HPTLC	-	High Performance Thin-Layer Chromatography
H_2O_2	-	Hydrogen peroxide
H_3PO_4	-	Orthophosphoric acid
HRP	-	Horseradish-peroxidase
HSE	-	Human skin equivalent
HSF	-	Human skin fibroblast
HTS	-	High throughput screening
IBD	-	Inflammatory bowel disease

IFN	-	Interferon
IFN-γ	-	Interferon-gamma
IL-1α	-	Interleukin 1-alpha
IL-1β	-	Interleukin 1-betha
IL-6	-	Interleukin-6
IL-8	-	Interleukin-8
IL-10	-	Interleukin-10
KI	-	Potassium iodide
LA	-	Lauric acid
LB	-	Lamellar bodies
LC	-	Langerhans cells
LCFA	-	long-chain fatty acid
LDF	-	Laser doppler flowmetry
LDL	-	Low-density lipoprotein
LED	-	Light-emitting diode
LEM	-	Leiden epidermal model
LN	-	Linoleic acid
LOX	-	Lipoxygenase
LPS	-	Lipopolysaccharide
IWP	-	Integrated wet process
IV	-	Iodin value
LC-MS	-	Liquid chromatography-mass spectroscopy
MA	-	Myristic acid
MCFA	-	Medium chain fatty acid
MCP-1	-	Monocyte chemoattractant protein-1
MCT	-	Medium chain triacylglycerol
mRNA	-	Messenger ribonucleic acid
MREC	-	Medical Research Ethic Committee
MS	-	Mass spectrometry
MSI	-	Multispectral imaging
MTT	-	3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide

MTX	-	Methotrexate
MUFA	-	Monounsaturated fatty acid
NADH	-	Nicotinamide adenine dinucleotide
NaOH	-	Natrium hydroxide
Na_2SO_3	-	Sodium thiosulfate
NF-kB	-	Nuclear factor-kappa B
NHS	-	Native human skin
NMF	-	Natural moisturising factor
NMR	-	Nuclear magnetic resonance spectroscopy
NO	-	Nitric oxide
NP	-	N-stearoyl phytosphingosine
NSB	-	Non-specific binding
NSAID	-	Non-steroidal anti-inflammatory drugs
NTS	-	Netherton syndrome
OA	-	Oleic acid
PA	-	Palmitic acid
PBS	-	Phosphate buffer saline
PDGF	-	Platelet derived growth factor
PF	-	Polyphenol
PG	-	Propylene glycol
PGE_2	-	Prostaglandin E_2
PL	-	Phospholipids
PUFA	-	Polyunsaturated fatty acids
PVA	-	Polyvinyl alcohol
RBD	-	Refined, bleached and deodorized
RCM	-	Reflectance confocal microscopy
RNA	-	Ribonucleic acid
ROS	-	Reactive oxygen species
RPL13A	-	Ribosomal protein L13A
RT-PCR	-	Reverse transcription-polymerase chain reaction
SB	-	Suction blister
SC	-	Stratum corneum

SCORAD	-	SCORing of Atopic Dermatitis
SCD	-	Stearoyl CoA desaturase
SEM	-	Scanning electron microscopy
SFA	-	Saturated fatty acid
SG	-	Stratum granulosum
SIS	-	Skin immune system
SLS	-	Sodium lauryl sulfate
SOD	-	Superoxide dismutase
SQ	-	Squalene
TAG	-	Triacylglycerol
TEWL	-	Transepidermal water loss
THP	-	Monocytes cells
TLC	-	Thin layer chromatography
TPC	-	Total phenolic content
TNF-α	-	Tumour necrosis factor-alpha
TS	-	Tape stripping
TG	-	Triglycerides
TO	-	Glyceryl tri-olein
ToF-SIMS	-	Time-of-flight secondary ion mass spectrometry
UFA	-	Unsaturated fatty acid
UPLC	-	Ultra-performance liquid chromatography
UV	-	Ultra violet
VLCFA	-	Very long-chain fatty acid
VOB	-	Vegetable oil blend
WAT	-	West African Tall
WB	-	Western blotting
WST	-	Water-soluble tetrazolium

LIST OF SYMBOLS

%	-	Percentage
° C	-	Degree celcius
α	-	Alpha
cfu	-	Colony forming unit
cm^2	-	Centimetre square
m^2	-	Metre square
µL	-	Micro liter
µg	-	Micro gram
g	-	Gram
h	-	Hour
IC_{50}	-	Median inhibitory concentration
LD_{50}	-	Median lethal dose
M	-	Molarity
mbar	-	Mili bar
meq	-	Mili equivalent
mL	-	Mili liter
mg	-	Mili gram
mm	-	Mili metre
mM	-	Mili molar
min	-	Minute
mJ	-	Mili joule
nm	-	Nano metre
ng	-	Nano gram
N	-	Normality
pg	-	Pico gram
p	-	Statistical significant
r	-	Pearson correlation coefficient
rpm	-	Rotation per minute

s	-	Second
Wt. %	-	Weight percentage
w/w	-	Weight/weight
w/v	-	Weight/volume
W	-	Watt

LIST OF APPENDICES

CHAPTER 1

INTRODUCTION

1.1 Research Background

The human body is protected from environmental factors such as harmful chemicals, air pollutants, and UV radiation by the skin. Direct skin exposure to ultraviolet (UV) light can cause several biological effects such as skin damage, skin cancer, and eye damage (Clydesdale, Dandie, and Muller, 2001). Chronic human skin exposure to UV radiation can lead to skin damage such as photoaging and photocarcinogenesis. Exposure to UVB (280-320 nm) is highly destructive to keratinocytes cells, contributing to DNA damage (Wang et al., 2014) and potential skin cancer. As a result of the UVB exposure, DNA-damaging reactive oxygen species (ROS) is generated along with the formation of DNA photolesions, predominantly cyclobutane pyrimidine dimers (CPDs) and (6-4) pyrimidine-pyrimidone (You et al., 2000). Pupe et al. (2002) reported that UVB irradiated skin keratinocytes activate the expression of various pro-inflammatory cytokines, such as tumour necrosis factor-α (TNF-α), interleukin-1α (IL-1α), and interleukin-6 (IL-6).

Stratum corneum (SC), the outer layer of the skin, acts as the main permeability epidermal barrier of the skin in preventing water loss and excreting various endogenous and exogenous chemicals (Lu et al., 2014). A change in the composition of SC lipids resulted in disrupted or weakened functions of the epidermal barrier, leading to increment in transepidermal water loss (TEWL) (Van Smeden et al., 2014a). If the skin is damaged by physical or chemical agents, an impairment in the epidermal barrier protecting activities function and TEWL enhancement can be seen. An increased TEWL value is observed in many skin diseases, such as atopic dermatitis (AD) and psoriasis (Takahashi et al., 2014; Borodzicz et al., 2016).

For centuries, virgin coconut oil (VCO) has been used traditionally in Southeast Asian countries as traditional medicine and skin health promotion. VCO has high nutritional value due to its antimicrobial, antioxidant, and anti-inflammatory effects (Intahphuak et al., 2010; Shankar, Ahuja, and Tracchio, 2013). VCO has been used to treat skin disorders such as AD (Kim et al., 2017; Wallace, 2019). A study revealed that VCO facilitates the provision of a better epidermal barrier protecting activities function by the native lipids in SC, and thus helps to moisturise the skin (Oyi et al., 2010). In addition, fatty acid (FA) components of VCO (caproic, caprylic, capric, lauric, myristic, palmitic, and stearic acids) contribute to its antioxidant properties (Kim et al., 2017). These components confer vital protection from sunburn, photoaging, and DNA degradation at the cellular level (Marina et al., 2009a, Kim et al., 2017). Furthermore, VCO was demonstrated to be effective in the elimination of free radicals that may induce skin inflammation. These antioxidant properties of VCO may also be attributed to phenolic compounds such as ferulic acid and *p*-coumaric acid (Marina et al., 2008).

In this study, VCO was processed with the integrated wet process (IWP) extraction using fresh coconut milk introduced by the Institute of Bioproduct Development (IBD) (Hamid et al., 2011). The coconut variety that was selected in this work is the West African Tall (WAT) which has been validated to produce the highest yield of VCO and contains the highest antioxidant and total phenolic compound (TPC) (Arlee et al., 2013; Nor Farahiyah, 2015). Previously, the traditional method used in the emulsion treatment for the production of VCO was found to be low-yields and time-consuming. The advantages of the process of IWP extraction are that is does not require any chemical agent or high temperature and also the emulsion treatment is rapid through the churning process. The high quality of VCO that was produced through the IWP process managed to preserve high yields of natural compounds such as essential FA, phenolic acids, and antioxidants (vitamin E) (Nur Arbainah, 2012). The IWP method was chosen to produce a high quality of VCO with higher antioxidant and phenolic compounds to be evaluated in the *in vitro* and *in vivo* anti-inflammatory and epidermal barrier protection activities.

To date, no trial assesses the comparison of VCO and refined coconut oil (RBD CO) for the *in vitro* anti-inflammatory effect on the epidermal barrier protection. The high nutritional value of VCO was compared with RBD CO to determine the effect of the phytochemical compounds such as FA derivatives, for example, myristic acid (MA) and palmitic acid (PA) on the *in vitro* anti-inflammatory activity in the human cellular model. The RBD CO was obtained from dried coconut meat (copra) that was extracted using the screw-press expeller machine in the dry extraction method and underwent the refining, bleaching, and deodorising processes (Nor et al., 2017; Wallace, 2019). The bioactive components (antioxidant and phenolic compounds) in VCO obtained from IWP extraction were superior to RBD CO as reported by Nur Arbainah (2012).

VCO was found to possess antinociceptive and anti-inflammatory agents by Zakaria et al. (2011) using various established *in vivo* animal models. A research was conducted by Intahphuak et al. (2010) on the anti-inflammatory, analgesic, and antipyretic properties of VCO using *in vivo* animal models. A recent study by Varma et al. (2019) has reported the effect of VCO in the *in vitro* anti-inflammatory activities on human skin cells such as keratinocytes (HaCaT), and human monocytes (THP-1). The study has investigated the effect of VCO on the inhibition of various pro-inflammatory cytokines after THP-1 were exposed to lipopolysaccharides (LPS) such as interferon-γ (IFN-γ), TNF-α, IL-6, interleukin-5 (IL-5), and interleukin-8 (IL-8). Besides, the *in vitro* skin irritation, UV protection, and phototoxicity potential of VCO were also studied. However, the effect of VCO and palm oil (PO) on the *in vivo* anti–inflammatory and epidermal barrier protection activities was not evaluated by Varma et al. (2019). Therefore, in this work, VCO and PO were discussed for *in vivo* anti-inflammatory and epidermal barrier protection activities studies.

In a recent study, Therese et al. (2014) reported the effects of topical VCO and mineral oil on paediatric patients with mild to moderate AD. The parameters evaluated in the study included SCORAD (SCORing of Atopic Dermatitis) index values, TEWL, and skin capacitance. The result showed that VCO was superior to mineral oil based on the clinical (SCORAD) and instrumental (TEWL and skin capacitance) assessments in which 46 % of the VCO test group showed an excellent improvement as compared to only 19 % in the mineral oil test group (Therese et al., 2014). The limitation of *in vivo* data for the effect of topically applied VCO on the human epidermal barrier protection activities such as the changes in SC lipid compositions (the fraction of ceramides (CERs), free fatty acids (FFAs), and cholesterol (CHOL) and skin biophysical measurement are yet to be elucidated. This data was not reported by Therese et al. (2014). A recent study by Pupala et al. (2019), showed that infants in the coconut oil group had reduced TEWL value with a better skin condition, lower infection rates, and higher growth rates.

In AD, a dysfunctional skin barrier can be indicated via the TEWL value (Denda, 2009). According to Danso et al. (2017), abnormalities in lipid compositions and organisation, for instance, reduction of relative CER content, shorter lipid chain length, and increment of unsaturated FAs fraction were observed in AD patients. In order to treat inflammatory conditions such as AD, many patients turn to the use of non-steroidal anti-inflammatory drugs (NSAID). Several steroidal and NSAID are used to treat skin inflammatory conditions via the inhibition of inflammation pathways. However, lifelong drug utilisation can contribute to various adverse effects such as skin cancer and skin thinning. Therefore, alternative treatment in the form of natural compounds such as VCO that can confer beneficial skin health effects should be explored.

1.2 Problem Statement

The potential of VCO as an anti-inflammatory agent in treating human skin diseases such as AD, psoriasis, and xerosis has gained increasing popularity recently. However, there is a very limited evidence on the benefits of VCO in protecting the skin from inflammation and epidermal barrier protecting disruption activities caused by UV radiation. Previously, the traditional method used in the emulsion treatment for the production of VCO is found to be limited and time-consuming; for example, in the fermentation extraction method. In this study, VCO was processed using the WAT coconut with the IWP extraction. The process of IWP extraction does not require any chemical agent or high temperature and the emulsion treatment is rapid and easy through the churning process. The IWP extraction can produce a higher yield of natural compounds such as phenolic acids and antioxidants (vitamin E) than the dry and wet processes (Nur Arbainah, 2012).

This study focused on the effects of VCO on the *in vitro* and *in vivo* anti–inflammatory and epidermal barrier protection activities on human skin cells (i.e. primary human keratinocytes and human dermal fibroblast cells). This study aimed to address this gap. The VCO produced from the IWP process using the WAT coconut has not been tested on the human cellular model and skin in terms of its efficacy in protecting the epidermal skin barrier protecting activities function. Therefore, to study the lipid profiles of human skin, the non-invasive tape stripping (TS) method was performed. In this study, the effect of skin barrier properties and epidermal lipid profiles between untreated and treated skin with VCO and PO was the main focus. PO was chosen as a comparison to VCO in the skin barrier protection study because it is widely used as topical emollients in cosmetic products in Malaysia. According to the European Union Cosmetic Products Regulations (2013), it is prohibited to market skin care products that have been tested on animals. Therefore, the TS method is used to collect the SC layer samples and to disrupt the SC layer in order to create a condition of epidermal barrier damage.

Furthermore, the function of the skin barrier, the biophysical properties of SC such as TEWL, and the skin hydration were also evaluated in this study. To the best of the researcher's knowledge, there is no published study on the effect of topical application of VCO and PO on human skin as well as the analysis of epidermal lipid (the fraction of CERs, FFAs, CHOL, etc.) using the TS and high-performance thin-layer chromatography (HPTLC) methods. Therefore, this HPTLC method was selected to analyse the skin lipid profiling because it has been validated to have a good accuracy and efficiency as well as the easiest method in this study.

1.3 Research Objective

The main objectives of this research are :

a) To investigate the anti-inflammatory properties of VCO extracted with the integrated wet extraction process with the physico-chemical properties and epidermal barrier protection activities.

b) To demonstrate the *in vitro* anti-inflammatory activity of VCO on human skin cell lines and in enzymatic assays.

c) To study the *in vivo* anti-inflammatory activity of VCO by analysing the skin biophysical properties and epidermal lipid composition on human skin treated with topical VCO.

1.4 Scope of the Study

In order to achieve the above objectives, several scopes have been identified as follows:

a) VCO was extracted from the WAT coconut using the IWP process under fixed operating conditions such as fixed temperature at 10 °C (chilled) and 37 °C (thawed), speed at 4000 rpm (centrifuged), and time about 20 minutes (centrifuged).

b) The physico-chemical and antioxidant properties of VCO were analysed such as the antioxidant activity and TPC using UV spectrophotometry, and the FA composition using gas chromatography (GC-FID). The titration method is used to determine the iodine, saponification, peroxide, and FFA values.

c) The cell cytotoxicity of VCO was investigated on the UVB-irradiated primary human keratinocytes and human epidermal fibroblast cell lines using the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay. The CO fatty acid derivatives such as MA and PA was also analysed for its toxicity on those cells.

d) The effectiveness of antioxidant properties in VCO to inhibit the intracellular reactive oxygen species (ROS) production in the UVB-irradiated human keratinocytes cells was analysed using 2',7'-dichlorofluorescein-diacetate (DCF-DA) assay. The ROS level in keratinocytes cells was also measured after the treatment with the MA and PA.

e) The *in vitro* anti-inflammatory potential of VCO was evaluated on the UVB-irradiated primary human keratinocytes cells using the ELISA pro-inflammatory assay for the detection of IL-6 and TNF-α. The anti-inflammatory effect of MA and PA also was identified in keratinocytes cells. Besides, the anti-inflammatory properties of VCO were determined using the enzymatic assays such as the inhibition of hyaluronidase (HAase) assay.

f) The wound healing potential of VCO was identified using the scratch-wound assay on the human epidermal fibroblast cell lines.

g) In the *in vivo* anti-inflammatory activities of VCO, the skin physiological changes on human volunteers treated with topical VCO and PO were investigated; for instance, TEWL, skin hydration, skin elasticity, skin melanin, and erythema index value using the multi-probe adapter system such as DermaLab® Combo and Cutometer MPA580. The skin lipid composition of human volunteers treated with topical VCO and PO was collected using the non-invasive TS method and analysed using the HPTLC.

1.5 Significance of the Study

This study provides a better understanding on the effects of VCO application towards the *in vitro* anti-inflammatory and epidermal barrier protection activities on human skin such as inhibition of the ROS and pro-inflammatory cytokines, such as TNF-α, IL-6 as well as proliferation and migration of human skin cells. Moreover, this research offers useful insight into the effect of topical application of VCO on human skin barrier recovery, especially after barrier disruption activities that alter the skin biophysical properties and the components of epidermal lipids in the SC layer. In short, this study provided a vital evaluation of the potential of VCO as a therapeutic agent in skin care product formulation for the treatment of dermatological disorders.

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Index Journal

1. Ahmad, Z., Sarmidi, M. R., and Hasham, R. (2017). Evaluation of Wound Closure Activity of *Cocos Nucifera* Oil on Scratched Monolayer of Human Dermal Fibroblasts. *Chemical Engineering Transactions*, 56, 1–6. https://doi.org/10.3303/CET1756277. (**Indexed by SCOPUS**)

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1. Ahmad, Z., Hasham, R., Nor, N. F. A., and Sarmidi, M. R. (2015). Physico-Chemical and Antioxidant Analysis of Virgin Coconut Oil Using West African Tall Variety. *Journal of Advanced Research in Material Science*, 13 (1), 1–10.

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